THE ULTRASTRUCTURE OF SPERMATOZOA OF THE AUSTRALIAN SKINKS, *CTENOTUS TAENIOLATUS*, *CARLIA PECTORALIS* AND *TILIQUA SCINCOIDES SCINCOIDES* (SCINCIDAE, REPTILIA).

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Spermatozoa of *Ctenotus taeniolatus*, *Carlia pectoralis* and *Tiliqua scincoides scincoides* are filiform, and approximately 84.0µm, 96.5µm and 57.5µm long, respectively. The acrosome vesicle is in the form of a hollow, concentrically zoned cone which basally overlies a paracrystalline subacrosomal cone which invests the tapered anterior end of the nucleus. The perforatorium is a slender rod extending anteriorly from the subacrosomal material. *Ctenotus* and *Tiliqua* resemble each other and differ from *Carlia* in the following respects: (1) acrosome elongate (relatively short in *Carlia)*; (2) acrosome depressed near its tip; (3) perforatorium strongly oblique (very slightly oblique in *Carlia*); (4) a conspicuous laminated structure on each side of the proximal centriole (absent in *Carlia*); (5) midpiece with four dense ring structures in longitudinal succession (in *Carlia* mitochondrial transformations are scattered irregular dense bodies of varying sizes); (6) mitochondria between the mitochondrial transformations form slightly sinuous columnar structures, in the order of 10 in transverse section, with numerous predominantly longitudinal cristae (in *Carlia* mitochondria are elongate, tubular structures, with indistinct cristae, which weave between the intermitochondrial bodies); (7) enlargement of the peripheral fibres adjacent to doublets 3 and 8 but not the gross enlargement which occurs in the anterior region of the axoneme in *Carlia* . In all three genera the midpiece terminates with an annulus; peripheral dense fibres are associated with the 9 triplets of the distal centriole and the doublets of the axoneme within the midpiece; only those peripheral fibres adjacent to doublets 3 and 8 remain conspicuous to the level of the annulus, each as a double structure associated with the annulated fibrous sheath; and all peripheral fibres are absent from the principal piece. The close similarity of the sperm of *Tiliqua* (in the *Egernia* group) to those of *Ctenotus* and *Nangura* (in the *Sphenomorphus* group) indicates that these groups form a monophyletic entity while the differences of the sperm of *Carlia* suggest that its Eugongylus group is less closely related. ☐ *Ctenotus taeniolatus, Carlia pectoralis, Tiliqua scincoides scincoides, Scincidae, Reptilia, spermatozoon, ultrastructure, phylogeny.*

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Published descriptions of the male gametes of the Scincidae are limited to: a description of the mature spermatozoon of *Chalcides ocellatus tiligugu* by Furieri (1970); an account of spermiogenesis, with some description of mature, epididymal sperm, in the same subspecies by Carcupino et al. (1989); a very brief account of the development of the midpiece in *Eumeces laticeps* by Okia (1990); and a description of mature sperm of *Nangura spinosa* (a genus and species recently erected by Covacevich, et al., 1993) by Jamieson & Scheltinga (1993). The latter paper lists all published references to the ultrastructure of spermatozoa or spermiogenesis in reptiles.

Greer (1979) separated the Australian Scincidae into three phylogenetic groups: The *Sphenomorphus* group (includes *Ctenotus*), *Eugongylus* group (includes *Carlia*) and *Egernia* group (includes *Tiliqua*).

MATERIALS AND METHODS

The testis and ducts of the Copper-tailed skink, *Ctenotus taeniolatus* (White, 1790) and the Rainbow skink *Carlia pectoralis* (De Vis, 1885) were dissected from a single euthenased specimen of each species. A portion of one duct was biopsied from a single specimen of Blue-tongued skink, *Tiliqua scincoides scincoides* (White, 1790), which was later released. *Ctenotus taeniolatus* and *Carlia pectoralis* were collected from Hervey Bay, Southeastern Queensland (SEQ). *T. scincoides* was collected from the Brisbane sub-

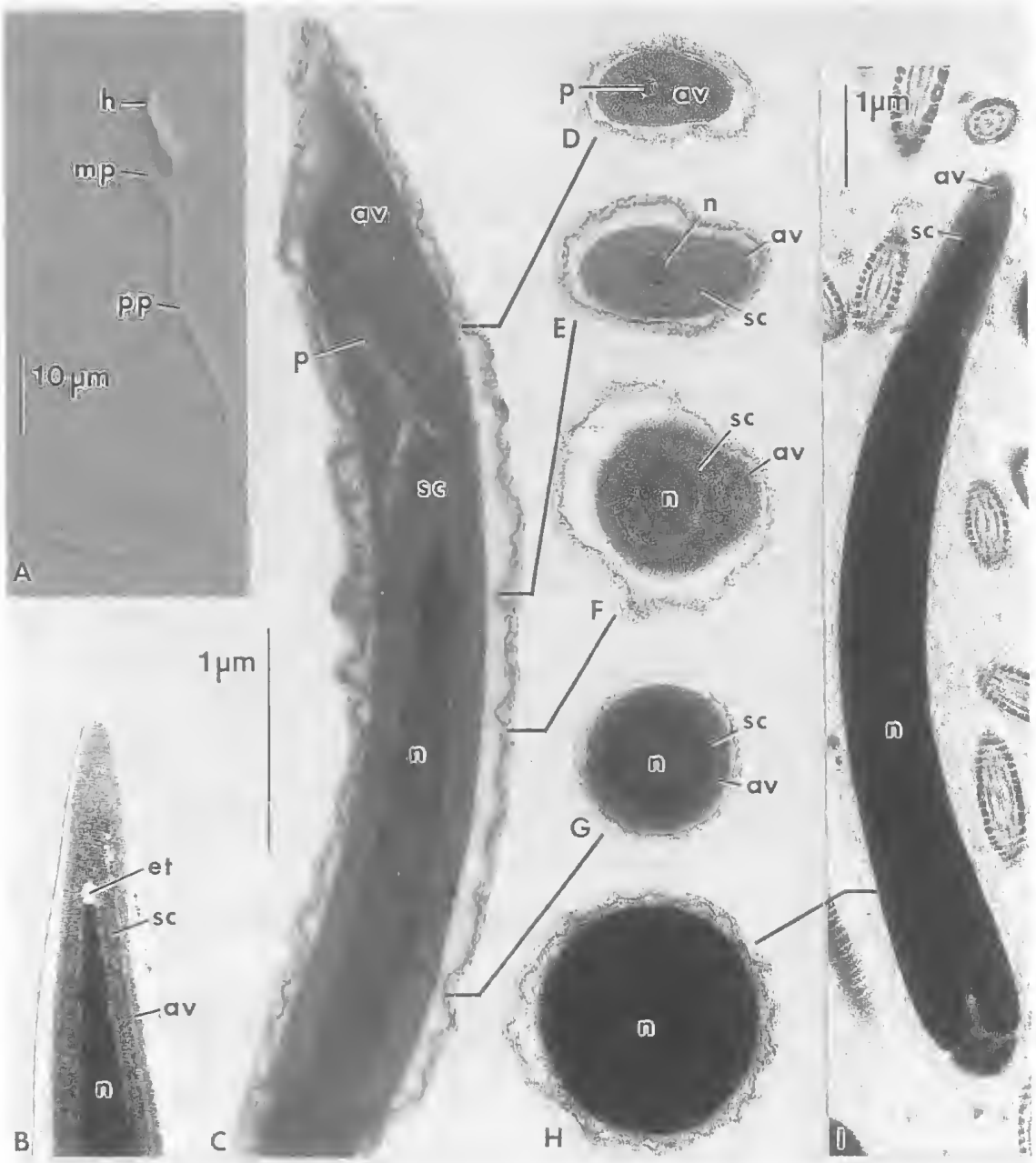


FIG. 1. *Ctenotus taeniolatus*. A, whole spermatozoon (Nomarski eontrast light microscopy); B, longitudinal section (L.S) through the nuelear point showing the epinuelear electron lueent region; C, L.S through the acrosome showing the nuelear point and that the longitudinal axis of the perforatorium appears to be slightly oblique relative to that of the acrosome vesiele; D-G, a series of transverse seetions (T.S) through the aerosome (Note that anteriorly, in D and E, the aerosomc is depressed in transversc sections, while further posteriorly, in F, it is unilatcrally ridged, and at its posterior limit, in G, it is circular); H, T.S through the nucleus. I, L.S through the length of the nucleus. B-H to the same scale, as indieated. Abbreviations: a = aerosome; av = acrosome vesiele; et = epinuelear electron lueent region; mp = midpiece; n = nueleus; p = perforatorium; pm = plasma membranc; pp = prineipal pieee; se = subaerosomal eone.

urb of Indooroopilly, SEQ. The tissues were diced into 1-2mm^3 portions, and fixed for transmission electron microscopy (TEM), in 3% glutaraldehyde in 0.1M sodium phosphate buffer (pH 7.2), at 4°C for 2 hours, being agitated for the first hour. The material was then rinsed in 0.1M phosphate buffer; post-fixed for 80 min in similarly buffered 1% osmium tetroxide; rinsed in buffer; dehydrated through an ascending ethanol series; and infiltrated and embedded in Spurr's epoxy resin. Sections were cut with diamond knives, on an LKB 2128 UM IV microtome. Thin sections, 50-80nm thick, were collected on carbon stabilized, colloidin-coated, 200µm mesh copper grids, rinsed in distilled water, stained for 30s in Reynold's lead citrate, then in 6% aqueous uranyl acetate for 1-4 mins and for a further 1-2 mins in lead citrate before final rinsing. Electron micrographs were taken on an Hitachi 300 electron microscope at 75kV and a JEOL 100-s electron microscope at 60kV. Light microscopic observations of spermatozoa, from glutaraldehyde-fixed tissue squashes, were made under Nomarski contrast using an Olympus BH2 microscope.

RESULTS

Spermatozoa of *Ctenotus taeniolatus*, *Carlia pectoralis* and *Tiliqua scincoides scincoides* are filiform, and approximately 84.0µm, 96.5µm (mean of 4, S.D = 7.1) and 57.5µm long (mean of 2, S.D = 3.6), respectively. Dimensions (for one or two sperm of *Ctenotus taeniolatus*, *Carlia pectoralis* and *Tiliqua scincoides scincoides*, respectively) are: 5.3µm, 2.3µm and 4.1µm for the length of the acrosome complex; 6.8µm, 2.9µm and 5.1µm for the nucleus posterior to the acrosome; 7.1µm, 11.5µm and 5.5µm for the midpiece, from transmission electron microscopy, and, from light microscopy 64.8µm, 79.8µm and 42.8µm for the flagellum behind the midpiece (principal piece) (Figs 1-6).

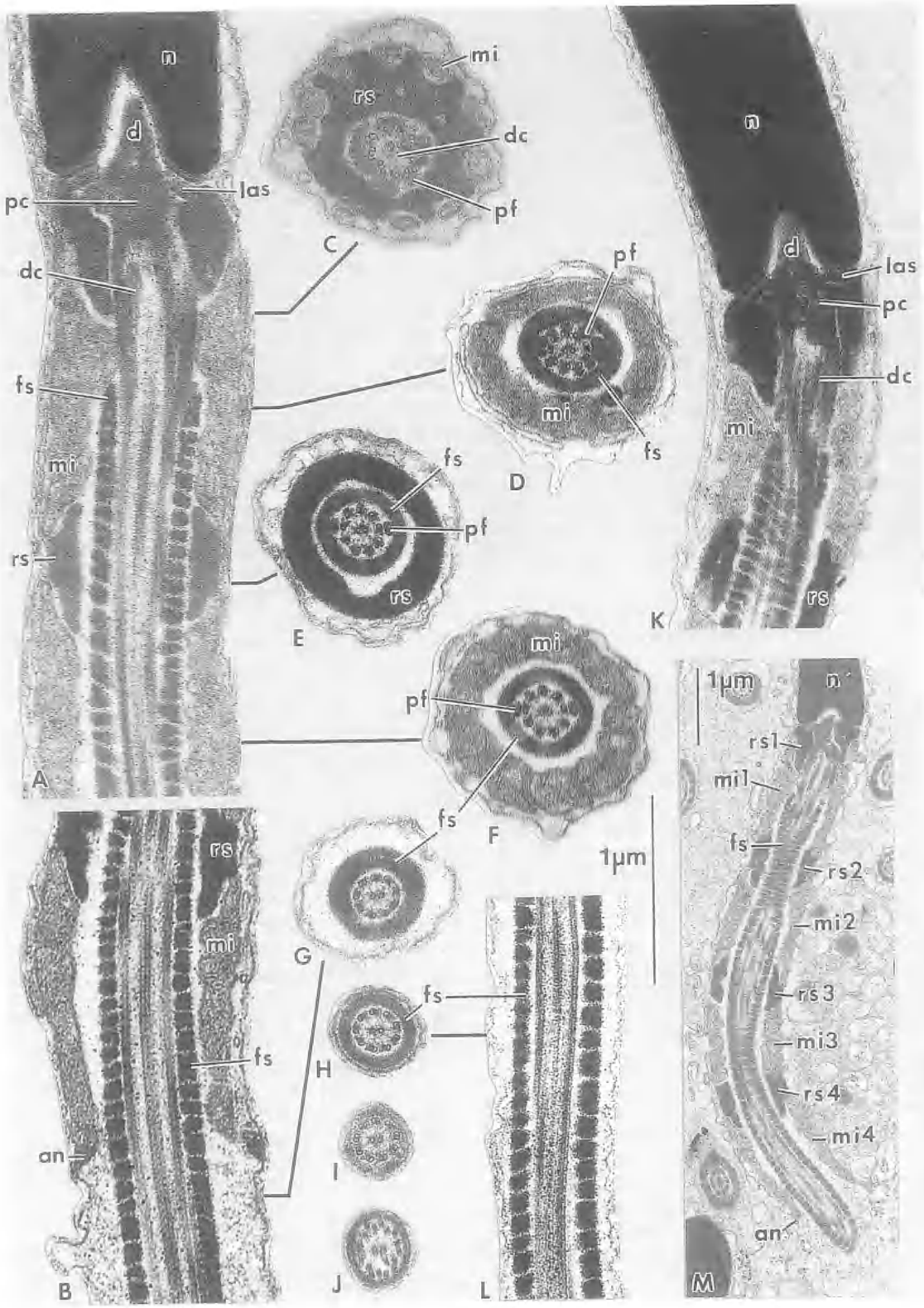
The head (acrosome and nucleus), and often the midpiece and flagellum, is curved (Figs 1A; 3A,B; 5A). As a result of this curvature in the spermatozoa of *Ctenotus* and *Tiliqua* it has not been possible to obtain a complete longitudinal section through the head. The sperm of all three skinks are circular in cross section with the exception of the acrosome of *Ctenotus* and *Tiliqua*. Although the acrosome of these two species is circular at its base, anterior to this *Ctenotus* sperm develops a unilateral ridge and from the tip of the nucleus it becomes increasingly depressed in the plane of curvature and elliptical in transverse section. The anterior region of the acrosome of *Tiliqua* becomes increasingly depressed and elliptical in transverse section. (Figs 1D-G; 5E-I; cf. *Carlia*, Figs 3E-J).

Acrosome Complex

The anterior end of the acrosome vesicle, comprising slightly less than half its total length, forms a thick walled hollow cone with a narrow lumen (subacrosomal space) housing the perforatorium (Figs 1C,D; 3C,E,G; 5B-F). The longer, posterior region of the vesicle is a thin walled continuation of this hollow cone, the vesicle here being no more than a sleeve-like investment (acrosome sleeve) of the subacrosomal material, as seen in longitudinal (Figs 1B,C,I; 3C; 5C) or transverse section (Figs 1E-G; 3H-J; 5G-I).

The perforatorium is a slender, moderately electron dense rod. In *Tiliqua* it shows some signs of internal longitudinal fibres (Fig. 5C). It extends anteriorly from the subacrosomal material, lying in the narrow subacrosomal space internal to the inner acrosome membrane (Figs 1C,D; 3C; 5B-D,F,K). In *Ctenotus* and *Tiliqua* the perforatorium extends through approximately the posterior half of the thick walled part of the acrosomal vesicle. A pale, central axial tube-like structure seen in *Ctenotus* (as in *Nangura spinosa*), which extends to the tip of the acrosome and displays some internal longitudinal fibres is not a forward continuation of the perforatorium. The perforatorium of *Carlia* extends from a perforatorial base plate (a pronounced basal swelling) to the apical tip of the acrosome (Fig. 3C). In all three species, the perforatorium makes contact at its posterior end with the subacrosomal material. Even allowing for the pronounced curvature of the acrosome of *Ctenotus* and *Tiliqua* the longitudinal axis of the perforatorium appears to be strongly oblique relative to that of the acrosome vesicle (Figs 1C; 5B,C) while that of *Carlia* is only very slightly oblique (Fig. 3C).

The material of the subacrosomal cone is paracrystalline, its matrix having fine obliquely longitudinal and less distinct transverse striations, indicating that it forms a fine lattice (Figs 1D,E,F,I; 3G,H; 5K). For most of its length, from its posterior end anteriorly, the subacrosomal cone invests the tapered anterior end of the nucleus (nuclear point). The nuclear point terminates within the anterior limit of the subacrosomal material at an epinuclear electron lucent region (Figs 1B; 3C,H; 5B). This region is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well defined in *Carlia* and to a lesser extent in *Ctenotus*, however in *Tiliqua* its presence is dubious.

In transverse sections of the acrosome vesicle anterior to the subacrosomal cone of *Ctenotus* and *Tiliqua* spermatozoa (Figs 1D; 5E,F), the vesicle is seen to have a concentric zonation which in sequence from the perforatorium outwards is: a narrow space around the perforatorium; a wide, dense, homogeneous zone; a narrow zone with radial striations; a thin, dense, homogeneous layer apposed to the plasma membrane. The narrow radially striated zone is not apparent in *Carlia*.

NUCLEUS

The nucleus is curved and tapers to a point within the basal region of the acrosome (acrosome sleeve). The transition from the tapered region (nuclear point) to the much longer cylindroid region is abrupt but the 'shoulders' seen in *Carlia* and many other reptile (and even *Ascaphus*) sperm are represented only by a gentle curvature on each side in *Ctenotus* and *Tiliqua*. The length of the nucleus from the base of the acrosome vesicle to the base of the nucleus of *Ctenotus taeniolatus*, *Carlia pectoralis* and *T. scincoides* respectively is 6.8µm, 2.9µm and 5.1µm with a further 2.8µm, 0.96µm and 1.9µm for the nuclear point which is surrounded by the subacrosomal cone (Figs 1C,I; 3B,C; 5B,C). The nucleus is almost parallel-sided, showing only a slight increase in width posteriad, from 0.64 to 1.0µm, 0.7 to 1.1µm and 0.57 to 0.9µm for *Ctenotus*, *Carlia* and *Tiliqua*, respectively, reaching its greatest width shortly before its posterior end. The cross section of the nucleus is circular throughout (Figs 1H; 3K; 5J; 6C). The chromatin is condensed and strongly electron dense. Basally the nucleus has a compact conical fossa which houses dense material extending from the proximal centriole (Figs 2A,K; 4A; 6A,K). The shape of the nuclear fossa varies from a dome shape in *Carlia* to a pointed cone in *Ctenotus*, with *Tiliqua* intermediate between these two states.

NECK REGION

The neck region (Figs 2A,K; 3D; 4A; 6A,K) is the region where the nucleus joins the midpiece and is recognized by virtue of its internal components although the anterior end of the midpiece directly abuts the posterior end of the nucleus. The neck region includes the proximal and distal centrioles and associated densities, including the first of the dense structures (mitochondrial transformations) of the midpiece. Each centriole consists of 9 triplets. The proximal centriole lies immediately anterior to the distal centriole and with its long axis at slightly less than a right angle to it (Figs 2A,K; 4A; 6A).

The long axis of the distal centriole, which forms the basal body of the flagellum, is in the long axis of the axoneme. In *Ctenotus* and *Tiliqua* the centrioles do not lie in the basal nuclear fossa but the proximal centriole, immediately behind this, is surmounted by a hollow conical density (dense cone) which conforms in shape with the nuclear fossa which it occupies. An electron lucent space separates it from the wall of the fossa. Compact dense material extends from the base of the dense cone to cover the more axial end of the proximal centriole and insinuates itself as a large mass between the proximal and distal centrioles (Figs 2A,K; 4A; 6A,C). In *Carlia* (Fig 3D) the proximal centriole is surrounded by a narrow zone of dense material but there is no conical extension of this; the anterior portion of the centriole is situated in the wide and shallow nuclear fossa and is closely apposed to the nucleus. In all three species two central singlets of the axoneme extend anteriad at least into the region of transition between the distal centriole and the axoneme. Unlike the proximal centriole, the distal centriole appears to always contain two central singlets, although longitudinal sections suggest that these are absent from its extreme anterior end. There is a density connecting triplet

FIG. 2. *Ctenotus taeniolatus*. A, longitudinal section (L.S) through the neck region showing, as a squamate autapomorphy, that the fibrous sheath penetrates the midpiece almost to the junction between the axoneme and the distal centriole; B, L.S through the midpiece-principal piece junction showing the annulus; C, T.S through the distal centriole-axonemal transition showing the 9 peripheral fibres associated with the triplets or doublets; D, T.S through the midpiece showing 9 peripheral fibres associated with the doublets; E, T.S through a ring structure; F, T.S through the midpiece showing 10 mitochondria surrounding the axoneme; G, H, T.S through the principal piece; I, J, T.S through the endpiece; K, L.S through the neck region; L, L.S through the principal piece; M, L.S through the midpiece showing the four ring structures and annulus separated by four sets of columnar mitochondria. A-L to the same scale, as indicated. Abbreviations: an = annulus; d = dense cone; dc = distal centriole; fs = fibrous sheath; las = laminar structure; mi = mitochondria; n = nucleus; nf = nuclear fossa; pc = proximal centriole; pf = peripheral fibre; pm = plasma membrane; rs = ring structure.

3 with the adjacent central singlet in addition to the peripheral dense fibre connected to each triplet or doublet (Figs 2C; 4C; 6E,F). The peripheral dense fibres are detached in some sections, indicating the commencement of the corresponding longitudinal column.

A conspicuous stratified laminar structure is seen in *Ctenotus* and *Tiliqua* to form a wing-like projection on each side of the proximal centriole near its anterior limit and is continuous around its axial pole (Figs 2A,K; 6A,D,K). It is therefore seen in some longitudinal profiles of the sperm, which are parallel to but not through the long axis of the axoneme, as a continuous wide lamina spanning much of the width of the nucleus behind the nuclear fossa (Fig. 6M). It is deduced that the lamina forms a thick disc around the proximal centriole but that the disc is interrupted at the peripheral end of the proximal centriole. Evidence for this interpretation is also seen in some transverse sections of the neck through the proximal centriole (Fig. 6M). In *Ctenotus* and *Tiliqua*, the outer edges of the laminar structure make contact with the first of the dense 'ring structures' of the midpiece, which are described below (Figs 2A,K; 6A,K) as does the peripheral end of the proximal centriole (Fig. 6D).

MIDPIECE

The midpiece includes the neck, described above. It consists of mitochondria, mitochondrial transformations (ring structures or dense bodies) and the contained axoneme with its fibrous sheath and ends posteriorly with the annulus.

In *Ctenotus* and *Tiliqua* the mitochondrial transformations are in the form of four ring structures (rs 1-4) in longitudinal succession, posterior to which lies the much smaller annulus. The ring structures, with the annulus, are separated by mitochondrial regions (mi 1-4). In terms of the pattern recognised for the teiid lizard *Cnemidophorus* by Newton & Trauth (1992), the formula for *Ctenotus* and *Tiliqua* are rs1/mi1, rs2/mi2, rs3/mi3, rs4/mi 4, an. Each ring structure appears in longitudinal section as an approximately kidney-shaped density on each side of the fibrous sheath of the axoneme (Figs 2A,M; 6A,B,M). In *T. scincoides*, as in *Nangura*, but less noticeably in *Ctenotus taeniolatus*, the profile of a ring structure on one side is often staggered relative to that on the other, though always overlapping it. This indicates that each ring structure is tilted relative to the axonemal axis (Figs 2A,M; 6A,B,M). In *Ctenotus taeniolatus* tilting is negligible. The mitochondrial transformations of *Carlia* are seen as scattered irregular dense bodies of varying sizes (Figs 3D; 4A-E,J).

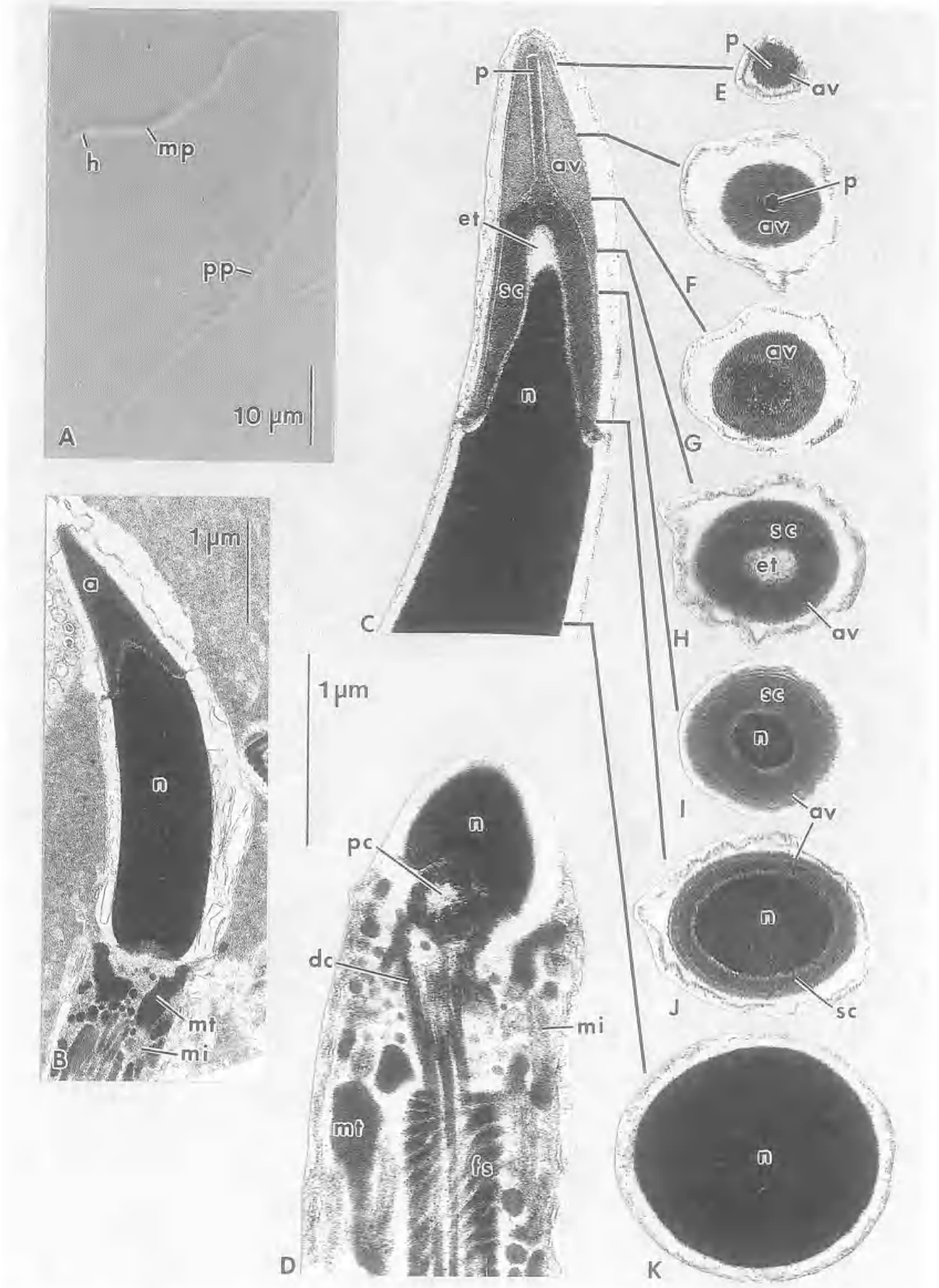
The mitochondria of *Ctenotus* and *Tiliqua* mostly form elongate, slightly sinuous columnar structures, with numerous predominantly longitudinal cristae, each of which extends from one ring structure to the next (Figs 2A,B,K,M; 6A,B,K,M). There are 10 or more (*Ctenotus*), or 9 or more (*Tiliqua*) mitochondria around the axoneme as seen in transverse section (Figs 2F; 6I). Occasional single, ovoid mitochondria are seen. Small mitochondria are often present lateral to the ring structures, the outer surface of which is then scalloped by them (Figs 2C; 6G).

The mitochondria of *Carlia* are elongate, tubular structures, with longitudinal cristae, which weave between the intermitochondrial bodies (Fig. 4A-E, J). In transverse section more than 25 mitochondria can usually be seen (Fig. 4E).

The axoneme has the usual pattern of nine doublet and two central singlet microtubules.

FIG. 3. *Carlia pectoralis*. A, whole spermatozoon (Nomarski contrast light microscopy); B, L.S through the nucleus; C, L.S through the acrosome showing the nuclear point, distinct nuclear 'shoulders', and acrosome; D, L.S through the neck region; E-J, a series of transverse sections (T.S) through the acrosome; K, T.S through the nucleus; C-K to the same scale, as indicated. Abbreviations: a = acrosome; av = acrosome vesicle; et = epinuclear electron lucent region; mp = midpiece; n = nucleus; p = perforatorium; pb = base plate of perforatorium; pm = plasma membrane; pp = principal piece; sc = subacrosomal cone; sh = nuclear 'shoulders'.

FIG. 4. *Carlia pectoralis*. A, longitudinal section (L.S) through the neck region showing that the fibrous sheath penetrates the midpiece almost to the junction between the axoneme and the distal centriole; B, L.S through the midpiece-principal piece junction showing the annulus; C, T.S through the distal centriole-axonemal transition showing the 9 peripheral fibres associated with the triplets or doublets; D, T.S through the midpiece showing the enlarged peripheral fibres at doublets 3 and 8; E, T.S through the midpiece showing the intermitochondrial bodies and mitochondria; F, Oblique T.S through the annulus; G, H, T.S through the principal piece; I, T.S through the endpiece; J, L.S through the length of the midpiece; K, L.S through the principal piece. A-I and K to the same scale, as indicated. Abbreviations: an = annulus; d = dense cone; dc = distal centriole; fs = fibrous sheath; las = laminar structure; mi = mitochondria; n = nucleus; nf = nuclear fossa; pc = proximal centriole; pf = peripheral fibre; pm = plasma membrane; rs = ring structure.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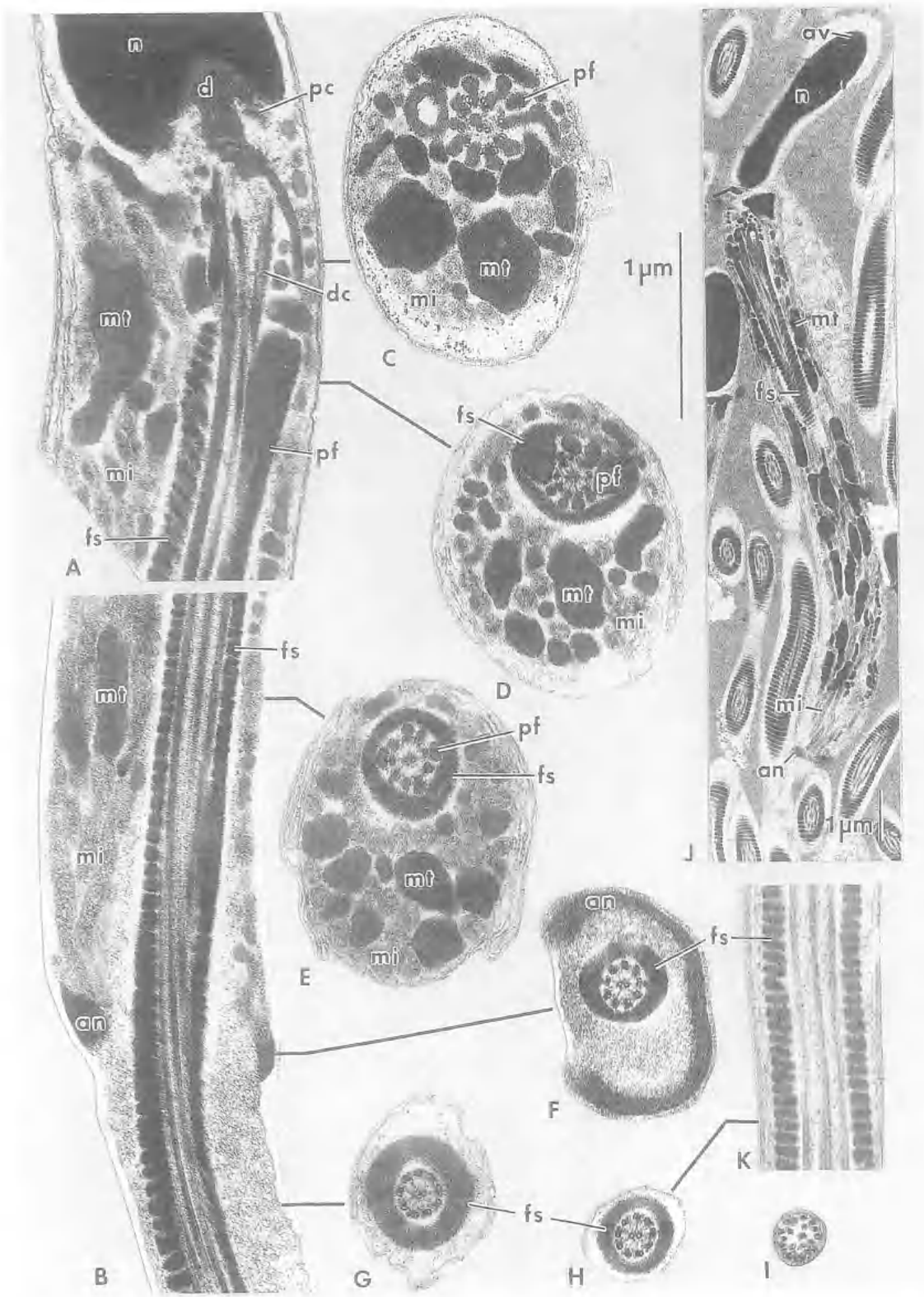
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Each doublet has two dynein arms. The A subtubule is occluded by dense material. Around the axoneme almost as far anteriorly as its junction with the distal centriole, there is a fibrous sheath (Figs 2A,K,M; 3D; 4A; 6A,K,M). In longitudinal section (Figs 2A,L; 4K; 6B,L) the fibrous sheath exhibits regularly arranged, approximately square to oblong dense blocks which, from glancing longitudinal sections (Figs 2M; 4J; 6M) and transverse sections (Figs 2D-H; 4D-H; 6G-J) are shown to form rings around the axoneme. They show a tendency to tilt relative to the axonemal axis and there are interruptions in the cross sections but that they form a spiral is questionable. Occasional anastomoses of adjacent rings are seen in tangential longitudinal sections.

Nine large peripheral dense fibres are associated with the transition between the distal centriole and the axoneme (Figs 2C-D; 4C-D; 6E-F) and continue posteriorly, though much narrower, along the axoneme into the midpiece (Figs 2E-F; 4E; 6G-I). One is attached externally to each triplet or doublet. Within the midpiece, at an undetermined level, all but two of the peripheral fibres become greatly reduced in size. Only peripheral fibres adjacent to doublets 3 and 8 remain conspicuous, as a double structure which for most of its length is nearer the fibrous sheath than it is to its doublet (Figs 2F; 6I). At the anterior end of the fibrous sheath in *Carlia* the peripheral fibres adjacent to doublets 3 and 8 are greatly enlarged, for a short distance, compared to *Ctenotus* and *Tiliqua* (Figs 2D; 4D; 6G). Peripheral fibres are seen in longitudinal section to be cross striated (Fig. 6A). In all three species, as in *Nangura spinosa*, the only well developed, though small, peripheral fibres at the level of the annulus are the double fibres at doublets 3 and 8 (Fig. 4F); at the beginning of the principal piece all nine dense fibres are already vestigial or absent (Figs 2G; 4G); and they are absent from the remainder of the principal piece (Figs 2H; 4H; 6J).

The annulus (Figs 2B,M; 4B,F,J; 6B,M) is a small dense ring with an irregular oval cross section. It is closely applied to the inner surface of the plasma membrane.

Principal Piece

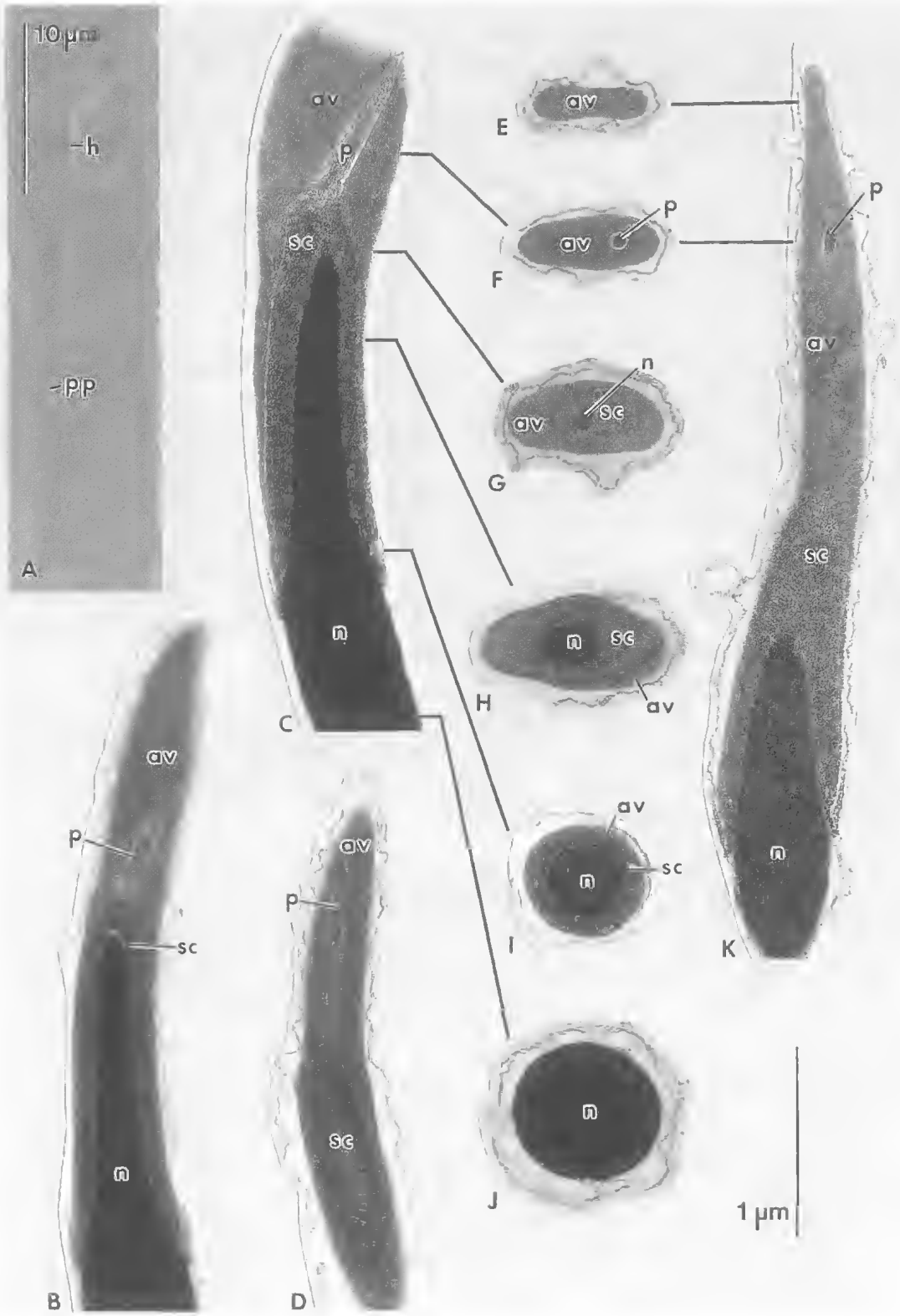
The principal piece, the longest part of the spermatozoon, consists of the continuation, behind the midpiece, of the axoneme with its surrounding fibrous sheath and plasma membrane. It begins, immediately behind the annulus, with a short region in which a wide zone of cytoplasm intervenes between the fibrous sheath and the plasma membrane (Figs 2B,G; 4B,G; 6B). The cytoplasm is finely granular, giving the region some resemblance to a glycogen piece but the presence of glycogen has not been determined. Posterior to this the plasma membrane is closely approximated to the fibrous sheath (Figs 2H,L; 4H,K; 6J,L).

Endpiece

The axoneme projects behind the fibrous sheath as an endpiece of undetermined length (Figs 2I-J; 6I). Within it the arrangement of microtubules becomes disrupted (Fig. 4I).

FIG. 5. *Tiliqua scincoides scincoides*. A, whole spermatozoon (Nomarski contrast light microscopy); B, L.S through the nuclear point; C, L.S through the acrosome showing the nuclear point and that the longitudinal axis of the perforatorium appears to be slightly oblique relative to that of the acrosome vesicle; D, L.S through the perforatorium; E-I, a series of transverse sections (T.S) through the acrosome. (Note that anteriorly, in E-H, the acrosome is depressed in transverse sections, while at its posterior limit, in I, it is circular); J, T.S through the nucleus; K, L.S through the length of the acrosome. B-K to the same scale, as indicated. Abbreviations: a = acrosome; av = acrosome vesicle; et = epinuclear electron lucent region; mp = midpiece; n = nucleus; p = perforatorium; pm = plasma membrane; pp = principal piece; sc = subacrosomal cone.

FIG. 6. *Tiliqua scincoides scincoides*. A, longitudinal section (L.S) through the neck region showing, that the fibrous sheath penetrates the midpiece almost to the junction between the axoneme and the distal centriole; B, L.S through the midpiece-principal piece junction showing the annulus; C, T.S through the nuclear fossa; D, L.S through the proximal centriole; E, F, T.S through the distal centriole-axonemal transition showing the 9 peripheral fibres associated with the triplets or doublets; G, T.S through a ring structure; H, T.S through the midpiece showing that the mitochondria may not completely surround the axoneme; I, T.S through the midpiece showing 9 mitochondria surrounding the axoneme; J, T.S through the principal piece; K, L.S through the neck region showing the proximal centriole in transverse section; L, L.S through the principal piece; M, L.S through the midpiece showing the four ring structures and annulus separated by four sets of columnar mitochondria. A-L to the same scale, as indicated. Abbreviations: an = annulus; d = dense cone; dc = distal centriole; fs = fibrous sheath; las = laminar structure; mi = mitochondria; n = nucleus; nf = nuclear fossa; pc = proximal centriole; pf = peripheral fibre; pm = plasma membrane; rs = ring structure.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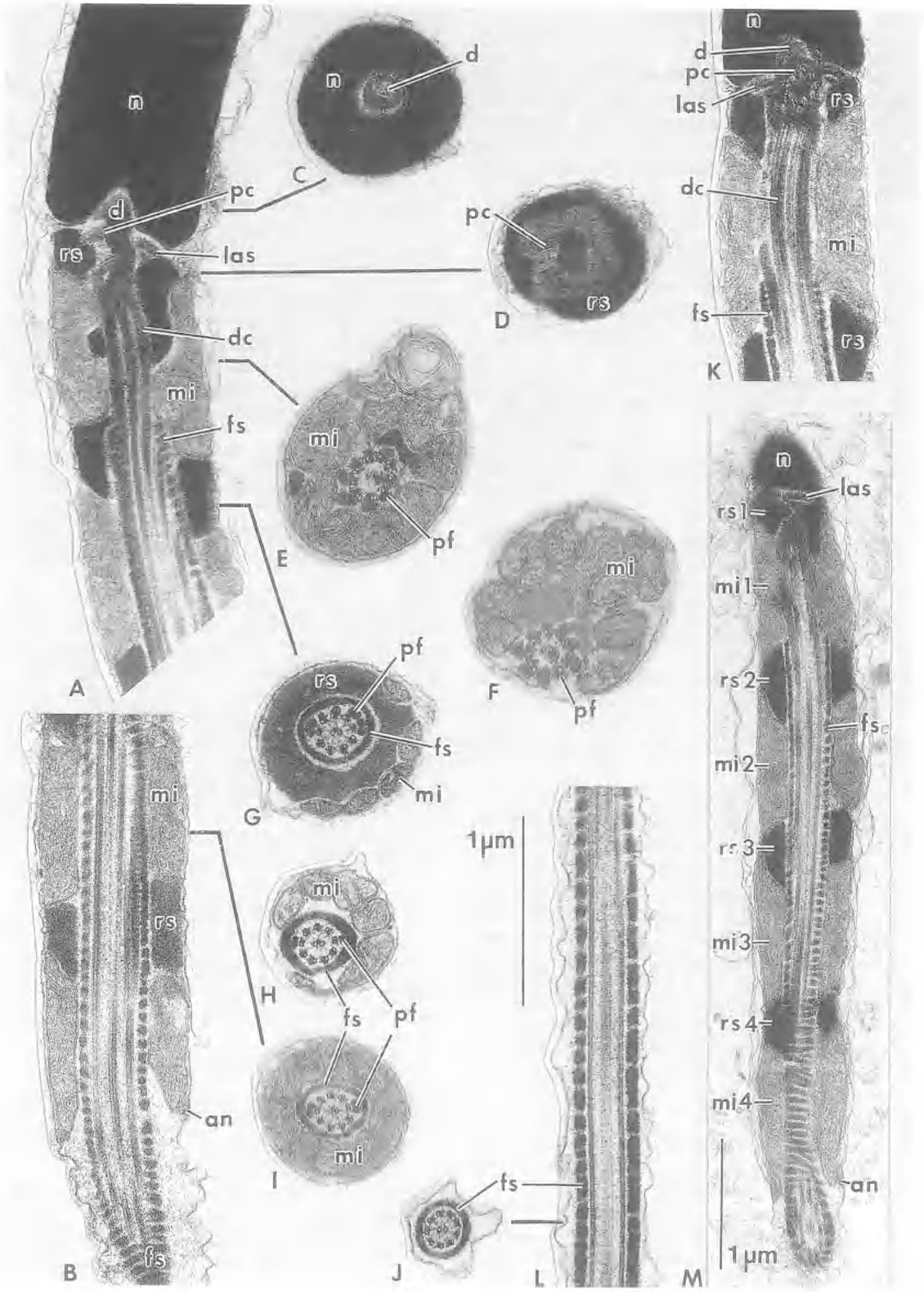
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DISCUSSION

Extension of the fibrous sheath into the midpiece seen in the sperm of the three species examined is an autapomorphy of the Squamata, unknown in the sperm of other reptiles or other amniotes (Healy & Jamieson, 1992; Jamieson & Healy, 1992).

Ctenotus taeniolatus is placed by Greer (1979) in the *Sphenomorphus* group. The sperm of *Nangura spinosa*, *Ctenotus robustus* and *Anomalopus verreauxii*, which are also in the *Sphenomorphus* group, have been examined by Jamieson & Scheltinga (1993; unpubl. data). *Carlia pectoralis* is included in the *Eugongylus* group of Greer (1979). Jamieson et al. (unpubl.) have examined the sperm of *Cryptoblepharus virgatus*, in the *Eugongylus* subgroup, and *Lampropholis delicata*, in the *Lampropholis* subgroup (this subgroup also includes *Carlia*). Greer's third group, the *Egernia* group, includes *Tiliqua scincoides scincoides*.

The species of the *Sphenomorphus* and *Egernia* groups are more similar to each other than they are to the *Eugongylus* group in possessing an anteriorly depressed acrosome and a sequence of ring structures in the midpiece. The species of the *Eugongylus* group differ in having scattered intermitochondrial bodies (considered homologous with the ring structures) in the form of small dense irregular spheres, tortuous rods or large plates and a shorter acrosome which is circular in transverse section.

The sperm of *Chalcides ocellatus tiligugu* examined by Furieri (1970) resemble *Ctenotus* and *Tiliqua* in having four regularly placed intermitochondrial rings but, unlike *Ctenotus* and *Tiliqua*, each ring is shown in a diagram, unsubstantiated by micrographs of transverse sections, to consist of a circlet of small juxtaposed spherules rather than a continuous ring. *Varanus gouldii flavirufus* also has 4 dense intermitochondrial structures in longitudinal sequence. However, each 'ring' is made of many loosely aggregated large granules that do not form a continuous ring (Oliver & Jamieson, unpublished). The sperm of the Lacertidae examined by Furieri (1970) also have intermitochondrial rings but only two are present, one around the proximal centriole, the other in the distal third of the midpiece.

A paracrystalline substructure of the subacrosomal cone, as here seen in skinks, has been recognized in other squamates (Butler & Gabri, 1984; Carcupino et al., 1989; Furieri, 1970). It is probably (Jamieson & Scheltinga, 1993) a synapomorphy, and autapomorphy, of the Squamata.

A dense ring, the annulus, at the posterior end of the midpiece is a feature of many metazoan sperm and is clearly plesiomorphic for amniotes. It has been demonstrated, inter alia, in turtles, crocodile, tinamou, Rhea, rooster, guineafowl, tuatara and monotremes. Squamates were considered exceptional in absence or at least negligible development of an annulus (Jamieson & Healy, 1992). However, an annulus has been demonstrated for *Lacerta vivipara* (see Courtens & Depeiges, 1985), *Cnemidophorus sexlineatus* (see Newton & Trauth, 1992) and for *Nangura spinosa* (see Jamieson & Scheltinga, 1993) and is possibly universal in squamates and, indeed, in reptiles, despite supposed absence in some accounts.

The number of mitochondria seen in transverse section of the midpiece, where possible near its anterior end, is very variable in amniotes. The number, approximately, 10 in *Ctenotus*, 9 in *Tiliqua* and 25 or more in *Carlia* is apomorphically high.

The intermitochondrial bodies, seen as 'ring structures' in *Ctenotus*, *Tiliqua* and *Nangura*, or irregular scattered dense bodies in *Carlia*, are limited to the squamates and were regarded as derivations of the dense bodies seen in basic amniotes (Jamieson & Healy, 1992). This derivation has received ontogenetic confirmation in the spermiogenesis of some squamates (Oliver & Jamieson, unpubl. data; Jamieson & Scheltinga, 1993). Carcupino et al. (1989), for *Chalcides ocellatus tiligugu*, independently concluded that the rings were mitochondrial derivatives.

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